Accurate and precise quantification of up to 8 samples simultaneously using the Qubit Flex Fluorometer

- The new Qubit Flex Fluorometer increases quantification throughput and capacity with the ability to measure up to 8 samples simultaneously
- The Qubit Flex Fluorometer improves the speed of processing quantification samples by up to 50%
- The Qubit family of fluorometers continues to have the intuitive user interface, calculators, and connectivity expected in today's modern laboratories

Introduction

Fluorescence and UV absorbance are the basis of two methods that are typically used to quantify DNA, RNA, and protein. The Invitrogen™ Qubit™ family of fluorescence-based quantification instruments has a new member: the Qubit™ Flex Fluorometer. Like the Invitrogen™ Qubit™ 4 Fluorometer, the Qubit Flex Fluorometer is a benchtop device designed for highly accurate quantification of DNA, RNA, and protein. Both the Qubit 4 Fluorometer and the new Qubit Flex Fluorometer use highly specific Invitrogen™ Qubit™ assay reagents, which contain a highly selective dye that emits fluorescence only when bound to the target molecule. Qubit assay reagents are available for dsDNA, ssDNA, RNA, and protein. Additionally, these optimized assays have been formulated to cover a broad concentration range of the target molecule.

The Qubit Flex Fluorometer increases quantification throughput with the ability to measure up to 8 samples simultaneously (Figure 1). The time it takes to generate quantification measurements compared to single-sample readers is significantly less, due in part to a 50% reduction in hands-on time. In addition to saving time, the ability to measure 8 samples simultaneously helps reduce variability, resulting in highly reproducible data.

Figure 1. The Qubit Flex Fluorometer increases throughput to quantify DNA, RNA, or protein from 1–8 samples simultaneously.

Materials and methods

Speed of quantification

To measure the time needed to quantify an increased number of samples, the Invitrogen™ Qubit™ 1X dsDNA HS Assay Kit (Cat. No. Q33231) was tested on the Qubit 4 Fluorometer and the Qubit Flex Fluorometer. The Invitrogen™ Qubit™ dsDNA HS Assay Kit (Cat. No. Q32854) was tested using another supplier's single-channel fluorometer that does not offer a 1X reagent solution workflow. Lambda DNA was diluted to 4 concentrations (0.1, 1, 5, and 10 ng/µL) and prepared and measured in replicates of 2, 6, 12, and 24 to obtain the desired final number of samples. The time required to prepare and quantitate 1, 8, 24, 48, and 96 samples on the Qubit 4 Fluorometer, Qubit Flex Fluorometer, and another supplier's single-channel fluorometer was recorded. The sample volume was 10 µL and the working solution volume was 190 µL.

Accuracy and precision of measurements

To test accuracy and precision, the Qubit dsDNA HS Assay Kit and the Invitrogen™ Qubit™ dsDNA BR Assay Kit (Cat. No. Q32853) were used with the Qubit 4 Fluorometer, the Qubit Flex Fluorometer, and another supplier's fluorometer. The samples were lambda DNA diluted to four concentrations (0.1, 1, 5, and 10 ng/µL for the dsDNA HS kit, and 2, 20, 50, and 100 ng/µL for the dsDNA BR kit). Samples were run in replicates of eight in individual Invitrogen™ Qubit™ Assay Tubes (Cat. No. Q32856) on two Qubit 4 Fluorometers and another supplier's fluorometer. Samples were run in replicates of eight in a single Invitrogen™ Qubit™ Assay Tube Strip (Cat. No. Q33252) on two Qubit Flex Fluorometers. The sample volume was 10 µL and the working solution volume was 190 µL. Samples were run according to the assay protocols. The percent deviation and percent coefficient of variation (CV) were calculated for each sample measurement and averaged across all concentrations for each instrument.

The percent deviation is used to determine the accuracy of a measurement. It is defined as:

$$\text{Deviation (\%)} = \left| \frac{(\text{expected} - \text{measured})}{\text{expected}} \right| \times 100$$

The percent CV is used to calculate the precision of a measurement. It is defined as:

$$\text{CV (\%)} = \left(\frac{\text{standard deviation}}{\text{mean}} \right) \times 100$$

Results

Speed of quantification

A time study showed a reduction of up to 50% in total time-to-data (including preparation and measurement of the samples) using the Qubit Flex Fluorometer, compared to a single-sample fluorometer for higher numbers of samples (Figure 2). Time savings are realized with batches of as few as 8 samples, and efficiency multiplies as the number of samples increases.

Accuracy and precision of measurements

The accuracy of quantification was assessed using the Qubit 1X dsDNA HS Assay Kit with the Qubit 4 Fluorometer, Qubit Flex Fluorometer, and another supplier's fluorometer (Figure 3). The Qubit Flex Fluorometer was the most accurate, with a deviation of only 3.4%, followed by the Qubit 4 Fluorometer with a 4.5% deviation. The other supplier's fluorometer had lower accuracy, with a 7.8% deviation.

Similarly, the precision of quantification was evaluated using the Qubit dsDNA BR Assay Kit with the Qubit 4 Fluorometer, Qubit Flex Fluorometer, and another supplier's fluorometer. The CV for all samples was measured for each data point and averaged across all concentrations for each instrument. The Qubit Flex Fluorometer measurements had the lowest percent CV, indicating higher precision.

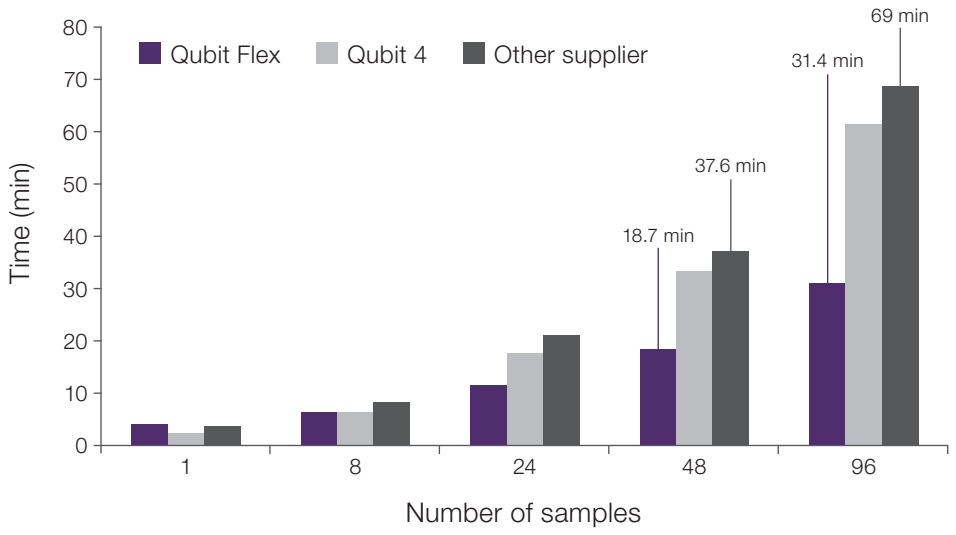

Figure 2. The Qubit Flex Fluorometer reduces time-to-data by up to 50%. A time study comparing the Qubit Flex Fluorometer to the Qubit 4 Fluorometer and another supplier's fluorometer, using the Qubit 1X dsDNA HS Assay Kit, showed time-to-data reduced by up to 50% with up to 96 samples.

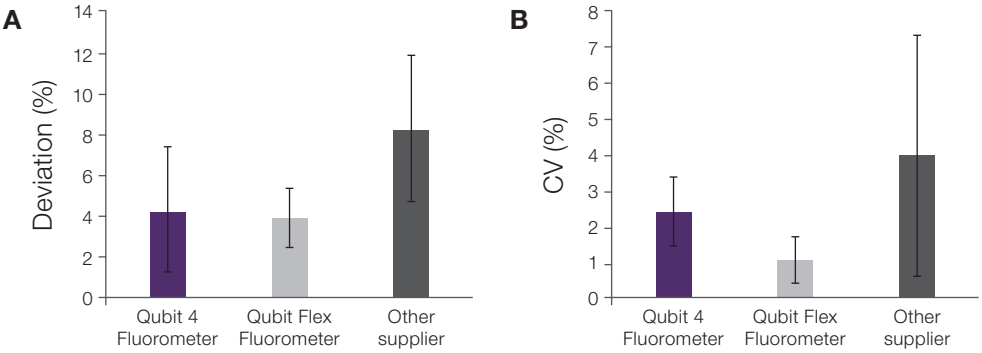

Figure 3. The Qubit Flex Fluorometer maintains accuracy and precision with increased throughput. (A) The low deviation demonstrates the accuracy of the Qubit Flex and Qubit 4 Fluorometers. **(B)** The Qubit Flex Fluorometer demonstrates the highest precision, having the lowest CV.

Discussion

When laboratories need to quantitate an increased number of samples due to changes in applications or scope of a project, a single-sample quantification reader is not only tedious but also a bottleneck in the workflow. The Qubit Flex Fluorometer was designed to address the growing need for medium-throughput laboratory capabilities while maintaining all the benefits of accuracy and reproducibility that have long been associated with Qubit fluorometers and assays.

The Qubit Flex Fluorometer generates concentration data based on the relationship between the 2 standards used in calibration (3 for the protein assay). Figure 4 shows the line corresponding to the (proprietary) curve-fitting algorithm used in the calculation of concentration for the Qubit RNA HS assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line. This plot demonstrates that the curve-fitting algorithm gives accurate values for quantification.

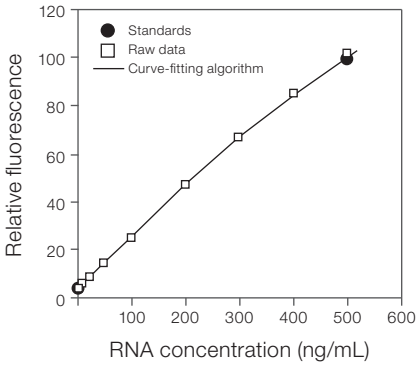

Figure 4. Concentration data for the Qubit RNA HS assay was calculated using a curve-fitting algorithm. The positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line.

Often the amount of sample is limited, and the Qubit Flex Fluorometer with Qubit assays requires only 1–20 µL of sample. The advanced optics and data analysis algorithms built into the Qubit Flex Fluorometer were designed and optimized to work together with the Qubit reagents, resulting in a seamless solution that generates highly reliable, sensitive, and specific results. All this innovation results in an optimized system that generates accurate measurements simply and quickly on your benchtop.

The Qubit Flex Fluorometer has built-in calculators for common calculations and to aid in determining assay volumes. The Reagent Calculator helps you determine how much working solution to prepare for the number of samples (Figure 5).

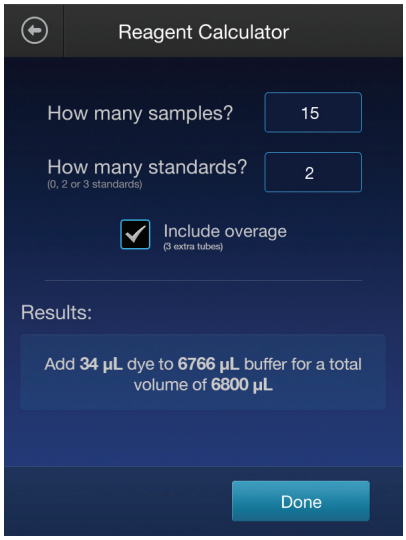

Figure 5. The Reagent Calculator can be used to determine master mix volumes for samples and standards.

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To generate accurate and precise results, it is important to select the best assay to use. The Assay Range Calculator displays the core sample concentration range for which the selected assay is most accurate, as well as the extended low and high ranges, based on your sample volume (Figure 6).

To aid in various downstream applications, Molarity and Normalization Calculators are included on the Qubit Flex Fluorometer. The Molarity Calculator allows you to calculate the molarity of a sample based on nucleic acid length and the measured concentration.

The Normalization Calculator replaces the need to transfer the data to a spreadsheet used to normalize samples during library preparation for sequencing. The results are easily normalized to a desired mass, concentration, or molarity.

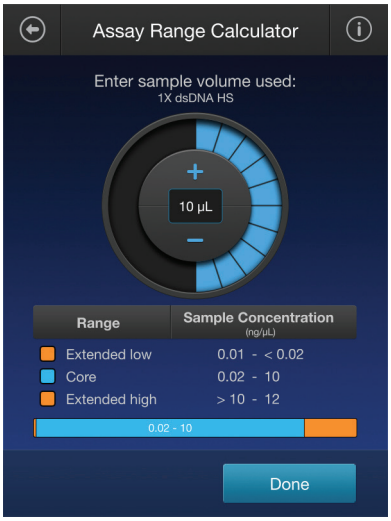

Figure 6. Assay Range Calculator. Choose the Qubit assay that will offer the best accuracy for the amount and concentration of sample available.

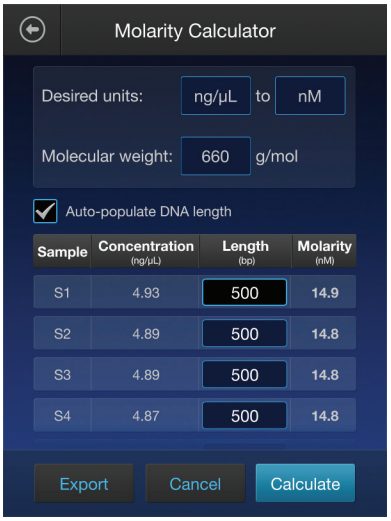

Figure 7. Molarity Calculator. Use the Molarity Calculator to quickly convert the length of nucleic acid and concentration to molarity.

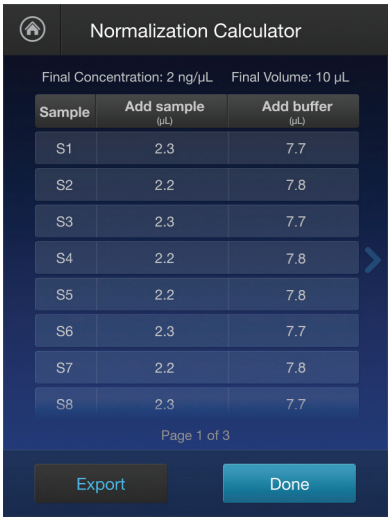

Figure 8. Normalization Calculator. The Normalization Calculator quickly provides the amount of sample and buffer needed to normalize to a final concentration and volume. It can easily be transferred via Wi-Fi, USB, or directly to a computer via USB cable.

The experiments outlined here demonstrate that the Qubit Flex Fluorometer improves the speed of processing quantification samples by up to 50% without compromising the accuracy and precision of the measurements. Researchers are continually being asked to do more (higher throughput) with less (time and sample); the Qubit Flex Fluorometer is designed with these demands in mind. Furthermore, the Qubit family of fluorometers continues to have the intuitive user interface, calculators, and connectivity expected in today's modern laboratories.

Find out more at **thermofisher.com/qubitflex**

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